Sexing Sub-adult Human Remains with Osteological and DNA-Analysis based Methodology

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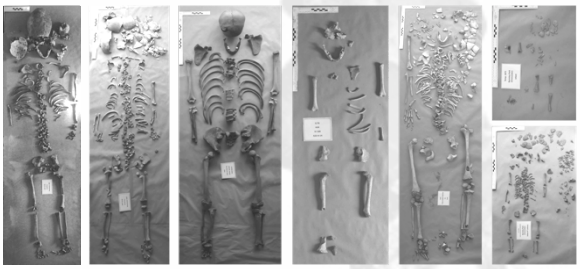
Within the forensic context the ability to sex skeletal remains reliably is of utmost importance in order to ascertain the identity of an individual. Contrary to the widespread assumption that it is impossible to sex the skeletal remains of sub-adults reliably, due to the constant developmental changes (Cardoso and Saunders 2008, Wilson *et al.* 2008), it is indeed possible that juvenile skeletal remains may be sexed and findings of sexual dimorphisms in the sub-adult skeletons have been published for over a century (Thomson 1899, Boucher 1957, Sundick 1977) and various methods to sex immature human skeletal remains reliably have been published (Boucher 1957, Weaver 1980, Hunt 1990, Scheuer 2002, De Vito and Saunders 1990, Schutkowski 1993, Loth and Henneberg 2001, Rogers 2009). The pelvic girdle, the mandible and the dentition are described as the most promising areas for the detection of sexual dimorphisms, though long bones also lend themselves for identification.

In order to establish the reliability of the employed methods for the sex determination in individuals when applied by researchers with limited experience in osteological analysis of skeletal remains six previously published methods assessing different skeletal areas were employed to assess the sex of the skeletal remains of seven sub-adult humans of the Gloucester Museum collection. In addition molecular sex determination was carried out typing Amelogenin and SRY.

Seven sub-adult skeletons at a variety of developmental stages and ages where at our disposal (figure below).



The specimen. Left to right: 1967.39.B5, 1967.39.B6, A.8206.8206a, 5/78 HSR, RI/G-TFI, Temp. 1871 (top) and No Number (bottom)

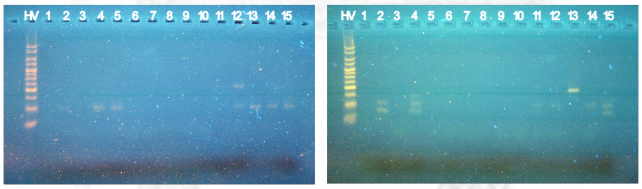
Preliminary results could be obtained for three specimen after the assessment with the methods previously published by Boucher (1957), Black (1978), Weaver (1980), Schutkowski (1993), Loth and Henneberg (2001) and Rogers (2009).

Osteological assessment of skeletal traits. M = male, F = female, U = undetermined); no traits with accuracy < 70%; Boucher not taken into account

Specimen	Sex
A.8206.8206a	U
1967.39.B6	U
1967.39.B5	F
5/78 HSR	U
RI/G-TFI	U
Temp. 1871	M
No number	F

All methods with an accuracy of less than 70% as stated in the publications were excluded in order to reach the preliminary results, so were the results of the Boucher (1957) method for being biased towards the male range.

For DNA analysis approximately 1g of bone from rib ends or long bones were removed, the surfaces cleaned, decontaminated and the samples ground to a fine powder. DNA was extracted following a Chelex protocol and precipitation after 70 hours decalcification of 0.1g in EDTA (0.5M, pH 8.3) (Schmerer in prep.). DNA extracts were amplified at the sexing loci Amelogenin and SRY. PCR conditions were 60 cycles following initial denaturation at 95°C for 5 minutes; 95°C for 30 seconds, 50°C for 1 minute and 72°C for 1 minute. Gel electrophoresis was done in 3.5% agarose gel, WebGreen or EtBr stained and 4.5% PAGE, EtBr stained.



Amelogenin and SRY PCR products. 3.5% Agarose gel, EtBr stained; HV = Hyperladder V; 1, 3, 5-9 = bone extracts; 2, 3, 10 = extraction blanks; 11 = pos. control ♀; 12 = pos. control ♂; 13-15 = no template controls

Initial amplifications showed the presence of remaining inhibitors in the extracts from the historical remains.

The assessments based on the selected methods show contradictory results for four of the specimen, despite the exclusion of all traits with less than 70% accuracy, while the other three specimen were sexed in agreement. Discrepancies in the accuracies stated can be found between the original publications and later reviews of the methods of Boucher (Weaver 1980), Weaver (Hunt 1990, Mittler and Sheridan 1992, Wilson *et al*. 2008), Schutkowski (Sutter 2003, Cardoso and Saunders, Wilson *et al.* 2008) and Loth and Henneberg (Scheuer 2002). As the osteological methods depend on the interpretation of the observer, discrepancies are expected.

PCR inhibition is a common problem encountered when amplifying ancient DNA, due to impurities in the extracts, which inhibit Taq Polymerase (Höss and Pääbo 1993, Schmerer 1999). Due to time restraints the DNA analysis could not be repeated, therefore a statistical evaluation of the methods employed as outlined in the objective could not be accomplished.

We like to thank the Gloucester Museum and Gallery for kindly providing us with the historical skeletal remains analysed here.

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